

WE CLAIM:

1. An isolated nucleic acid molecule that encodes a PPO polypeptide of lettuce, banana, tobacco or pineapple wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:
 - (i) a nucleotide sequence selected from the group consisting of: SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29;
 - (ii) a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of: SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, and 30;
 - (iii) a fragment of (i) or (ii) comprising a nucleotide sequence that encodes the copper-binding site of a PPO polypeptide;
 - (iv) a degenerate nucleotide sequence of (i) or (ii) or (iii); and
 - (v) a nucleotide sequence that is complementary to (i) or (ii) or (iii).
 2. The isolated nucleotide sequence according to claim 1 wherein the copper-binding site is the CuA binding site of said banana, tobacco or pineapple PPO polypeptide.
 3. The isolated nucleotide sequence according to claim 1 wherein the copper-binding site is the CuB binding site of said banana, tobacco or pineapple PPO polypeptide.
 4. The isolated nucleic acid molecule according to claim 1, wherein the PPO polypeptide of banana is at least expressed in banana peel.
 5. The isolated nucleic acid molecule according to claim 1, wherein the PPO polypeptide of tobacco is at least expressed in tobacco leaves.
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6. The isolated nucleic acid molecule according to claim 1, wherein the PPO polypeptide of pineapple is at least expressed in pineapple fruit.
 7. A recombinant vector comprising the isolated nucleic acid molecule

according to claim 1 inserted within a vector molecule.

8. The recombinant vector according to claim 7 wherein the vector is a plasmid expression vector.

9. The recombinant vector according to claim 8 wherein the plasmid expression vector is Bluescript SK+.

10. The recombinant vector according to claim 7 wherein the vector is a binary vector suitable for introducing into a plant cell, tissue or organ.

11. The recombinant vector according to claim 7, wherein the vector is capable of being replicated and the PPO-encoding nucleotide sequence is capable of being transcribed and translated in a unicellular organism or in a plant.

12. A method of increasing the level of banana, pineapple or tobacco PPO activity in a plant or a cell, tissue or organ thereof, said method comprising:

(a) introducing a nucleotide sequence to said plant or a cell, tissue or organ thereof which nucleotide sequence encodes a PPO polypeptide of banana, tobacco or pineapple and is selected from the group consisting of:

(i) a nucleotide sequence selected from the group consisting of: SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29;

(ii) a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of: SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, and 30;

(iii) a fragment of (i) or (ii) comprising a nucleotide sequence that encodes the copper-binding site of a PPO polypeptide;

(iv) a degenerate nucleotide sequence of (i) or (ii) or (iii); and

(v) a nucleotide sequence that is complementary to (i) or (ii) or (iii); and

(b) expressing said nucleotide sequence to produce an enzymatically-active PPO polypeptide.

13. A method of decreasing the level of PPO activity in a plant or a cell, tissue or organ thereof, said method comprising introducing a nucleic acid molecule to said plant or a cell, tissue or organ thereof which comprises a nucleotide sequence selected from the group consisting of:

- (i) a nucleotide sequence selected from the group consisting of: SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29;
- (ii) a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of: SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, and 30;
- (iii) a fragment of (i) or (ii) comprising a nucleotide sequence that encodes the copper-binding site of a PPO polypeptide;
- (iv) a degenerate nucleotide sequence of (i) or (ii) or (iii); and
- (v) a nucleotide sequence that is complementary to (i) or (ii) or (iii).

14. The method according to claim 13 further comprising expressing the introduced nucleic acid molecule to produce sense or antisense RNA therefrom.

15. The method according to claim 13 wherein the PPO activity is decreased by co-suppression of the endogenous PPO-encoding genes that would otherwise be expressed in the plant or a cell, tissue or organ thereof.

16. The method according to claim 13, wherein the PPO activity is decreased by the expression of antisense RNA that is complementary to RNA encoded by an endogenous PPO-encoding gene that would otherwise be expressed in the plant or a cell, tissue or organ thereof.

17. The method according to claim 12 wherein the nucleic acid molecule is introduced into the plant or a cell, tissue or organ thereof by means of ~~Agrobacterium~~-mediated transformation.

18. The method according to claim 13 wherein the nucleic acid molecule is introduced into the plant or a cell, tissue or organ thereof by means of ~~Agrobacterium~~-mediated transformation.

19. The method according to claim 12, wherein the nucleic acid molecule is introduced into the plant or a cell, tissue or organ thereof by means of microparticle bombardment using a nucleic acid-coated microprojectile.

20. The method according to claim 13, wherein the nucleic acid molecule is introduced into the plant or a cell, tissue or organ thereof by means of microparticle bombardment using a nucleic acid-coated microprojectile.

21. A transformed plant comprising the isolated nucleic acid molecule according to claim 1 or a plant part, progeny or propagule thereof that also comprises said nucleic acid molecule.

22. A transformed plant comprising the recombinant vector according to claim 7, or a plant part, progeny or propagule thereof that also comprises said nucleic acid molecule.

23. An isolated nucleic acid molecule that encodes a PPO polypeptide of lettuce, banana, tobacco or pineapple wherein said nucleic acid molecule is isolated by a method comprising:

(i) providing:

(a) banana, lettuce, tobacco or pineapple PPO cells, tissue or organs having PPO activity;

(b) a first primer having a nucleotide sequence capable of hybridizing to a copper (Cu) binding site-encoding region of a PPO gene selected from the group consisting of: SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29 or upstream thereof;

(c) a second primer having a nucleotide sequence capable of hybridizing to the complement of a copper (Cu) binding site-encoding region of a PPO gene selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, or downstream thereof; and

(d) an adaptor primer;

(ii) isolating RNA from said cells, tissues or organs;

- (iii) treating the RNA to construct copy DNA (cDNA) therefrom; and
- (iv) amplifying the cDNA so formed using the first and second primers.

24. The isolated nucleic acid molecule according to claim 23 wherein the first primer comprises a nucleotide sequence selected from the group consisting of:

- (i) 5'-GCGAATTCTT[TC][TC]TICCITT[TC][CA][TC][AC]G-3' (SEQ ID NO: 31);
- (ii) 5'-GCGAATTCGATCCACITT[TC]GC[GT]TTICC-3' (SEQ ID NO: 32);
- (iii) 5'-GCGAATTCAA[TC]GTIGA[TC][AC]GIATGTGG-3' (SEQ ID NO: 33);
- (iv) 5'-GCGAATTCTICA[TC]TG[TC]GCITA[TC]TG-3' (SEQ ID NO: 34);
- (v) 5'-GCGAATTCTTICCIT[TA][TC]TGGAAT[TC]TGGG-3' (SEQ ID NO: 35); and
- (vi) a hybridizable fragment of any one of (i) to (v).

25. The isolated nucleic acid molecule according to claim 23 wherein the second primer comprises a nucleotide sequence selected from the group consisting of:

- (i) 5'-GCCTGCAGCCACATC[TG][AG]TCIAC[AG]TT-3' (SEQ ID NO: 36);
- (ii) 5'-GCCTGCAGTT[TC]TC[AG]TC[AG]TAGAA-3' (SEQ ID NO: 37); and
- (iii) a hybridizable fragment of (i) or (ii).

26. The isolated nucleic acid molecule according to claim 23 wherein treating the RNA to construct cDNA includes treating the RNA with reverse transcriptase and an adaptor primer that comprises the nucleotide sequence:

5'-GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3' (SEQ ID NO: 38)

or a hybridizable fragment thereof to form cDNA.

27. The isolated nucleic acid molecule of claim 23 wherein the method further comprises obtaining nucleic acid encoding the N-terminal fragment of the PPO polypeptide of banana, lettuce, tobacco or pineapple by attaching an anchor to the 5'-end of the cDNA formed and amplifying said cDNA using a first primer that binds to said anchor and a second primer in the antisense orientation, and wherein the nucleotide sequence of said second primer is derived from the sequence of the internal PPO fragment.

28. The isolated nucleic acid molecule of claim 23 wherein the method further comprises obtaining nucleic acid encoding the C-terminal fragment of the PPO polypeptide of banana, lettuce, tobacco or pineapple by amplifying said cDNA using an adaptor primer and a primer in the sense orientation, and wherein the nucleotide sequence of said second primer is derived from the sequence of the internal PPO fragment.

29. The isolated nucleic acid molecule according to claim 28 wherein the primer in the sense orientation comprises the nucleotide sequence:

5'-CGCTGGGTGGGTAATTCTAGGATG-3' (SEQ ID NO: 46)

or a hybridizable fragment thereof.

30. The isolated nucleic acid molecule according to claim 28 wherein the primer in the sense orientation comprises the nucleotide sequence:

5'-AGTCATCCACAATGCGGCGCACATG-3' (SEQ ID NO: 47)

or a hybridizable fragment thereof.

31. The isolated nucleic acid molecule according to claim 28 wherein the primer in the sense orientation comprises the nucleotide sequence:

5'-GTTGCTCTTCTTAGGCTCGGCTTAC-3' (SEQ ID NO: 48)

or a hybridizable fragment thereof.

32. The isolated nucleic acid molecule according to claim 27 wherein the primer in the antisense orientation comprises a nucleotide sequence selected from the group consisting of:

(i) 5'-ATATCACCTGTCGGTACATGACGGC-3' (SEQ ID NO: 39);

(ii) 5'-GTGCCATTGTAGTCGAGGTCAATCA-3' (SEQ ID NO: 40);

(iii) 5'-CCAGTGCCTGGTTTAGGTGTATTCAC-3' (SEQ ID NO: 41); and

(iii) a hybridizable fragment of (i) or (ii) or (iii).

33. The isolated nucleic acid molecule according to claim 28 wherein the adaptor primer includes the following sequence or a hybridizable fragment thereof:

5'-GACTCGAGTCGACATCG-3' (SEQ ID NO: 49).

34. The isolated nucleic acid molecule according to claim 27 wherein the primer in the antisense orientation comprises a nucleotide sequence selected from the group consisting of:

- (i) 5'TGCTGTTCTGTTCTGAACATGGCAG-3' (SEQ ID NO: 42);
 - (ii) 5'-TATACAAGTGGCACCAGTGTCTGC-3' (SEQ ID NO: 43);
 - (iii) 5'-CCGCATTGTGGATGACTTCCATCTG-3' (SEQ ID NO: 44);
 - (iv) 5'-CCAGAATGGGATGGTGAAGGTGTCG-3' (SEQ ID NO: 45); and
 - (v) a hybridizable fragment of any one of (i) to (iv).
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